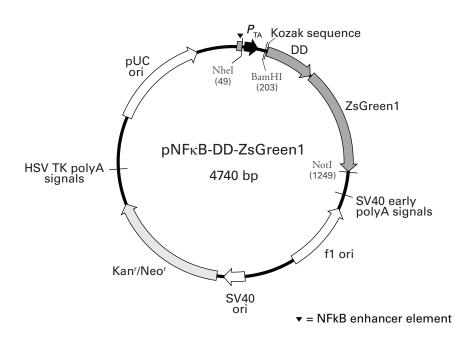
Sold as part of Cat. No. 631079



pNFkB-DD-ZsGreen1 Reporter Vector Map

# **Description**

pNFκB-DD-ZsGreen1 is a reporter vector that allows you to monitor NFκB activation in mammalian cells. The vector contains an NFκB enhancer element (composed of four tandem copies of the NFκB consensus binding sequence; 1) upstream of a minimal TA promoter ( $P_{\text{TA}}$ ), which consists of the TATA box from the herpes simplex virus thymidine kinase (HSV-TK) promoter. The vector encodes the reporter protein DD-ZsGreen1, a ligand-dependent, destabilized green fluorescent protein that minimizes background fluorescence from leaky promoters.

ZsGreen1 is a human codon-optimized variant of the reef coral *Zoanthus sp.* green fluorescent protein (ZsGreen) that has been engineered for brighter fluorescence (excitation and emission maxima: 493 and 505 nm, respectively; 1, 2). DD-ZsGreen1 is a modified version of ZsGreen1 that is tagged on its N-terminus with the ProteoTuner™ destabilization domain (DD; 3). The presence of this destabilization domain causes rapid, proteasomal degradation of the fluorescent fusion protein; however, when the membrane permeant ligand Shield1 is added to the medium, it binds to the destabilization domain and protects the fusion protein from degradation.

In the absence of Shield1, the destabilization domain causes the degradation of any DD-ZsGreen1 reporter protein produced prior to promoter activation, thus reducing background fluorescence. In order to analyze NF $\kappa$ B activation, an inducer of choice is added to the medium along with the Shield1 stabilizing ligand, which effectively stabilizes the reporter protein, allowing it to accumulate. As a result, only the reporter molecules expressed during promoter induction will contribute to the fluorescence signal, providing a considerably higher signal-to-noise ratio than that obtained with non-destabilized or constitutively destabilized reporter systems. The high signal-to-noise ratio also allows the monitoring of NF $\kappa$ B activation during discrete windows of time when Shield1 is added to the cell medium for discrete periods of time.



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The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo<sup>r</sup>) allows stably transfected eukaryotic cells to be selected using G418 (4). This cassette consists of the SV40 early promoter, aTn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSVTK) polyadenylation signals. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

#### Use

The pNFκB-DD-ZsGreen1 Reporter vector, available as part of the NFκB DD Green Reporter System (Cat. No. 631079), can be used to monitor NFκB activation in live cells as well as *in vivo*. pNFκB-DD-ZsGreen1 Reporter can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418.

### Location of features

NFκB enhancer element: 54–93
P<sub>TA</sub> (TA minimal promoter): 113–119

• Kozak sequence: 214-224

• DD-ZsGreen1

Start codon (ATG): 221–223; Stop codon: 1244–1246 DD (FKBP-L106P destabilization domain; 3): 221–544

ZsGreen1 (Zoanthus sp. green fluorescent protein): 551–1243

• SV40 early polyA signals: 1399–1433

• f1 origin of replication: 1496-1951 (complementary)

SV40 origin of replication: 2292–2430

• Kan<sup>r</sup>/Neo<sup>r</sup> (kanamycin/neomycin resistance gene)

Neomycin phosphotransferase coding sequences: 2476–3270

HSVTK polyA signals: 3506–3524
pUC origin of replication: 3855–4498

# Propagation in E. coli

- Recommended host strains: DH5 $\alpha^{\text{TM}}$ , HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in E. coli hosts.
- E. coli replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

# Excitation and emission maxima of ZsGreen1

- Excitation maximum = 493 nm
- Emission maximum = 505 nm

#### References

- 1. Pessara, U. & Koch, N. (1990) Mol. Cell Biol. 10(8):4146-4154.
- 2. Matz, M. V. et al. (1999) Nature Biotech. 17(10):969-973.
- 3. Haas, J. et al. (1996) Curr. Biol. 6(3):315-324
- 4. Banaszynski, L. et al. (2006) Cell 126(5):995-1004.
- 5. Gorman, C. (1985) In DNA Cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

**Note:** The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

Clontech Laboratories, Inc. www.clontech.com Protocol No. PT5116-5
2 Version No. PR9Z3417

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The RCFP's (including DsRedExpress and DsRedExpress2) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979 and 7,442,522.

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